Loring Pond Duckweed Abundance and Diversity Assessment Via By-Catch of Surface Skimming, and Suitability of Compost for Organic Gardens

Biology Undergraduate Research at Minneapolis Community and Technical College (MCTC)

Brianna Matchette

Abstract

Duckweed are tiny aquatic plants that are part of the Lemnaceae family including five genera: *Lemna, Landoltia, Spirodela, Wolffia*, and *Wolfiella*. Previous research has shown duckweed to have strong properties of phytoremediation of heavy metals and other pollutants (Ekperusi et al., 2019). Here we examine Loring Pond, an urban pond in Minneapolis, Minnesota, that is partially covered by duckweed, and the levels of biodiversity found in the surface skimming by-catch of this wetland. We also discuss the potential of utilizing composted duckweed in agriculture. Shallow marsh sites monitored across Minnesota, by the Minnesota Department of Natural Resources, have recorded H-index ranges of 2.7 – 3.6 (MN DNR Wetland Hydrology Monitoring Report 2023, p. 21). After identifying and quantifying the specimens observed during surveys conducted, and utilizing the Shannon-Wiener index, we show that diversity in the surface skimming by-catch of Loring Pond is low with determination in June, July, and August to be H = 0.388, 0.126, and 0.212, respectively, Overall, watermeal (*Wolffia spp.*) dominated surveys and indicated an inverse relationship with both biodiversity and certain heavy metal levels. Elemental analysis of composted duckweed revealed a few heavy metals fell below detectable limit, including Beryllium (Be), Cadmium (Cd), Vanadium (V), and Rubidium (Rb). Lead (Pb) was found to exceed allowable limits in organic fertilizers (27 mg/kg) 4 times

out of 7 samples and reaching just over 51 mg/kg at the highest level. Our results confirmed that wetlands covered by duckweed may have limited biodiversity, although surveys in this study revealed 15 genera total. Four other plant genera and eight animal phyla were observed, which suggests a variety of organisms and elements would be removed during commercial skimming of Loring Pond. Further testing is needed to confirm the ability of duckweed to inhibit diversity in the pond itself, along with the viability of utilizing composted duckweed in agriculture due to the persistence of Pb.

Introduction

Biodiversity is considered to be vital to the structure of an ecosystem and can be measured from the numbers of species that make up a biological community in a given area; this can indicate whether or not an environment is healthy and stable (Jewel et al., 2018). Wetlands provide habitats to thousands of species. The ecological function of a freshwater system relies on the nutrient content and chemical composition of the water itself (Ronkainen, 2021). Photosynthesis is the primary source of dissolved oxygen in many aquatic ecosystems, and depletion of dissolved oxygen causes anoxic conditions. Under non-eutrophic conditions, photosynthetically active radiation (PAR) can provide phytoplankton and other macrophytes with energy for photosynthesis. PAR is reduced when mats of aquatic plants are present, thus reducing total photosynthesis (Ronkainen, 2021), and possibly biodiversity.

Duckweed, comprised of five genera of aquatic plants from the family Lemnaceae, are free floating plants that commonly inhabit and often invade freshwater ponds and lakes by forming a thick blanket across the wetland. Reproduction occurs quickly for this family, typically asexually, by dividing to form separate individual plants. One study concluded that a single frond could produce as many as 10 generations of daughter plants over a period as little as 10 days before dying and another found that the plant doubles its mass in less than 2 days under ideal conditions (Ekperusi et al., 2019). This rapid reproduction forms mats across wetlands that can instigate eutrophic conditions by blocking sunlight at the surface level. Duckweed mats restrict photosynthesis in the water column, resulting in low total photosynthesis and ecosystems that are low in oxygen and high in phosphorus (Ronkainen, 2021).

While their abundance and ability to invade certain wetlands have been made clear, duckweed are also known for their properties of phytoremediation. Phytoremediation is the application of plants for the rectification of a polluted environment. Aquatic macrophytes have been widely used for wastewater treatments and the remediation of aquatic environments over the past 30 years (Ekperusi, et al., 2019). Duckweed has shown strong potentials for the phytoremediation of many pollutants, including but not limited to heavy metals such as lead (Pb) and copper (Cu), agrochemicals, and pharmaceuticals, with the common duckweed (*Lemna minor*) being seen as one of the most effective (Ekperusi, et al., 2019). Other potential uses of duckweed include biofuel and food for livestock and possibly humans, due to its high level of protein and amino acids (Ekperusi et al., 2019).

Pollution is one of the most critical existential problems affecting modern society. The potential of duckweed as a phytoremediator could provide relief to certain environments. While thick mats of free-floating plants create stressful aquatic environments, the removal of duckweed mats using herbicides or mechanical skimming, are often temporary, possibly detrimental to the environment, and are monetarily costly. The potential to remove and utilize duckweed as compost would resolve the issue of material and monetary waste. The aim in this study is to examine the pervasive duckweed, how it affects biodiversity in a wetland, and assess its potential use as composted material.

Methods

Sample Collection

Samples in this study were collected at Loring Pond, a shallow pond with an average depth of no more than 1.5 m. The same site at Loring Pond was used, in the northwest corner of the pond (approximate GPS coordinates: 44.970698, -93.286185). The site was sampled three times in the summer months of 2023; once on June 15th, July 27th, and August 31st. Sample collection was done by skimming the surface of the pond near the shoreline using a 5' wide floating skimming net with 0.25 inch mesh, pulled by a rope. Roughly half of each sample was composted outside while the remaining half was stored and refrigerated until surveys were completed.

Diversity Analysis

To accurately count number of *Lemna* and *Wolffia*, surveys were split into two groups based on size: > 5 mm and < 5 mm. For each survey, species of duckweed and other organisms were identified and quantified in samples taken from Loring Pond using a dissecting microscope. Organisms that were too large or created an obstructed view were placed back within the corresponding bag to be counted in the > 5 mm surveys. For this group, one survey was completed for each sample date where 400 mL of each sample was investigated for any organism over 5 mm. Each organism found was sorted then identified, and a total count was created.

For organisms < 5 mm, a 5 mm^2 grid system was utilized, and five surveys were done for each sample date. Each survey consisted of one spatula scoop of material weighing between 1.0 – 1.3g that was placed on the 5 mm^2 grid. A probe was used to slowly spread and thin the material to create one layer and avoid damaging any potential specimen. Five randomly selected grids were surveyed where organisms were identified and counted. Groups were created for unidentifiable and non-living specimen, including living non-woody (L/NW), non-living non-woody (NL/NW), and non-living woody (NL/W). The preceding information was used to calculate diversity via Shannon-Wiener Index and provide data for graphical analysis.

Elemental Analysis

To examine the nutrient content of duckweed, concentrations of 27 elements was obtained via elemental analysis of composted duckweed samples. This was performed by the University of Minnesota Research Analytical Lab, where the compost is digested using the EPA 3051 method and larger particle sizes (> 9.5mm) are sieved out before grinding the smaller particles for analysis. The Dumas method is used to estimate plant total Nitrogen. Information acquired by the compost digestion and previous nutrient level reports were utilized in comparisons made between Loring Pond and other urban wetlands, including those that are not pervaded by duckweed and watermeal.

Results

Diversity Analysis

Few species were found in both survey groups, with *Wolffia spp.* dominating the < 5mm group (see Table 1) and molluscs in the genus *Planorbella* dominating the > 5mm group (see

Table 2). Three aquatic plant genera were observed in the < 5 mm group: *Wolffia*, *Lemna*, and *Spirodela*, with total observations of 4387, 155, and 19 respectively. Overall observations of the three sample dates for *Spirodela* averaged 1.27, while *Lemna* and *Wolffia* averaged 10.33 and 292.47 respectively (Table 1). One insect family, *Chironomidae*, was noted only in the August surveys. Among the three surveys in the > 5 mm search, four plant genera were found along with the following phyla: two Arthropoda*,* four Mollusca, and one Annelida. Only *Planorbella* and *Physella* from phyla Mollusca appeared in all three samples (Table 2).

Table 1. Observed Species < 5mm

Species/Genus (common name)	Sample Date	Total # of Observations Across All Samples	Average # of Observations per Survey in June, July, August
Wolffia spp. (Watermeal)	6/15, 7/27, 8/31	4387	355, 218.6, 303.8
Lemna minor (Lesser duckweed)	6/15, 7/27, 8/31	155	8.4, 17, 5.6
Spirodela polyrhiza (Greater duckweed)	6/15, 7/27, 8/31	19	0.2, 2.2, 1.4
Chironomidae (Midge, larvae)	8/31	3	0, 0, 0.6
L/NW	6/15, 7/27, 8/31	16	0.2, 1.8, 2
NL/NW	6/15, 7/27, 8/31	32	0.4, 2.6, 3.4
NL/W	8/31	2	0, 0, 0.4

Table 2. Observed Genus and total number of observations

Genus (common name)	Sample Date	Total # of Observations
Planorbella (Ramshorn)	6/15, 7/27, 8/31	34
Physella (Bladder snail)	6/15, 7/27, 8/31	13
Discus (Disk snail)	7/27	1
Spharium (Fingernail clam)	7/27, 8/31	4
Coleomegilla (Aquatic lady beetle)	7/27	1
Belostoma (Giant water bug)	8/31	2
Glossiphoniidae (Snail leech)	8/31	1
Myriophyllum (Milfoil)	6/15, 8/31	3
Elodea (Waterweed)	6/15	1
Potamogeton (Curly-leaf pondweed)	6/15	1
Ceratophyllum (Hornwort)	8/31	1

Average observations of individual *Wolffia* plants decreased by roughly 136.4 from June to July and increased by 85.2 in August (see Figure 1 and 2). Conversely, average observations of both individual *Lemna* and *Spirodela* plants increased in July then decreased in August (see Figure 1 and 3). Average observations varied more for *Lemna* compared to *Spirodela*, with average observations of *Lemna* increasing just over 100% from June to July and decreasing over 100% from July to August. Size of individual *Lemna* and *Spirodela* plants were seen to be different during June and August surveys, though the difference was found to be statistically insignificant with a p-val of 0.23.

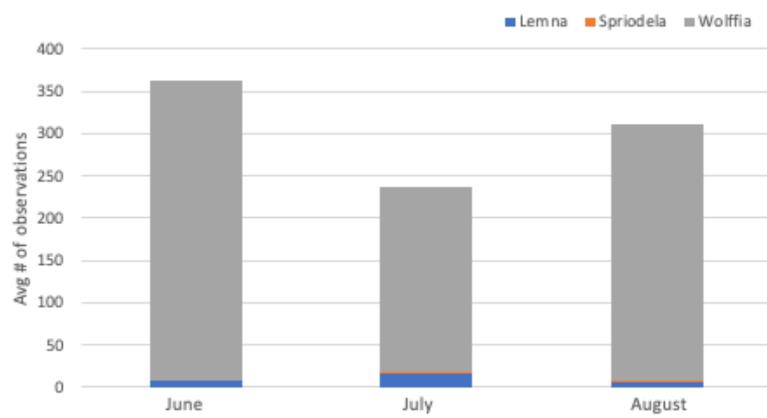

Fig 1. Average number of observations per survey for the < 5mm group.

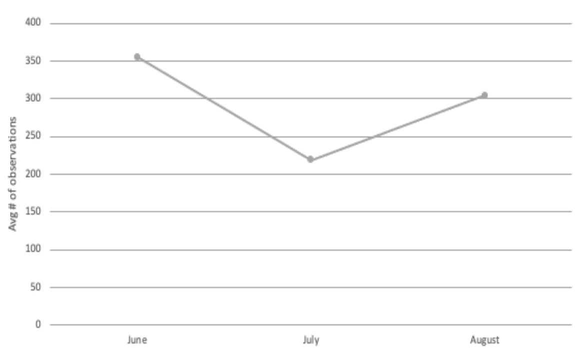

Fig 2. Average observations of *Wolffia* over time.

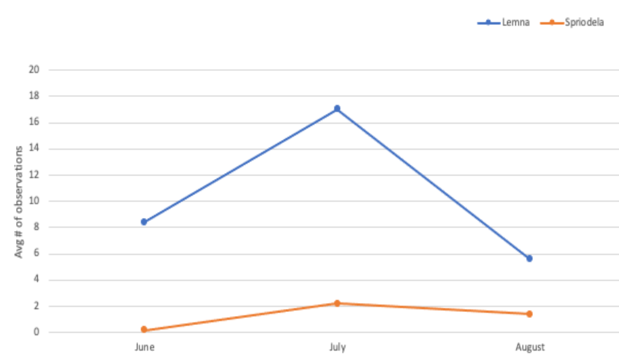

Fig 3. Average observations of *Lemna* and *Spirodela* over time.

Overall, diversity was found to be low in surveys < 5 mm from Loring Pond samples. Diversity was determined to be highest in July at 0.388 (s.d. = 0.03) compared to June at 0.126 (s.d. = 0.09) and August at 0.212 (s.d. = 0.05) (Figure 4).

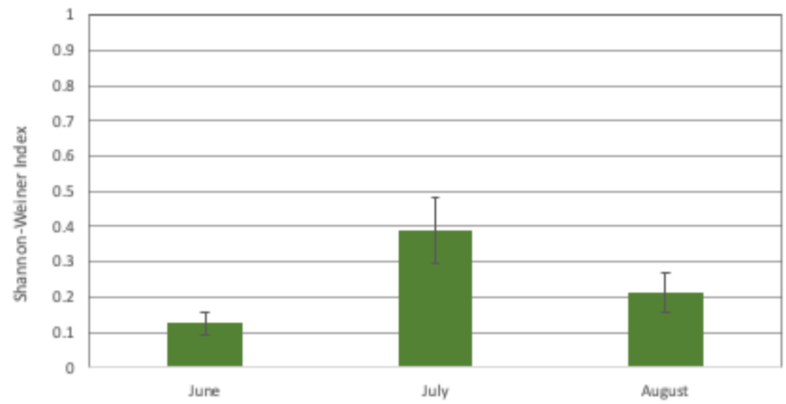

Fig 4. Survey (< 5 mm) diversity levels from Loring Pond.

Elemental Analysis

Elemental analysis of duckweed compost shows metals Be, V, and Rb levels fell below detectable limit in all samples. Cd can be seen to fall below the detectable limit all but twice, reaching no more than 0.4 mg/kg. Pb and Cu levels appear to be highest in July samples, reaching approximately 27 mg/kg and 51 m/kg respectively (see Table 3). Phosphorous levels continually decrease from June to August with highest levels reaching 8439.6 mg/kg and lowest levels reaching 4364.7 mg/kg. Total Nitrogen is found to increase from June to July and decreases from July to August (see Table 4).

Table 3. Elemental Analysis of Composted Duckweed

Laboratory ID	Sample ID	Al (mg/kg)	As (mg/kg)	B (mg/kg)	Ba (mg/kg)	Be (mg/kg)	Ca (mg/kg)	Cd (mg/kg)	Co (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	K (mg/kg)	Li (mg/kg)	Mg (mg/kg)
LOD (mg/L)**		0.014	0.008	0.007	0.001	0.001	0.444	0.001	0.001	0.001	0.012	0.009	0.155	0.001	0.208
LOD * average spl dilution factor (~mg/kg)		**4.48**	**2.56**	**2.24**	**0.32**	**0.32**	**142.08**	**0.32**	**0.32**	**0.32**	**3.84**	**2.88**	**49.60**	**0.32**	**66.56**
1	Jun 15 f	2104.5	2.823	387.60	209.19	<0.32	180582	<0.32	1.772	4.857	18.205	3972.7	32955	3.326	9193.1
2	Jun 15 d	1691.3	3.052	456.91	244.03	<0.32	176672	<0.32	1.527	4.150	12.128	3397.2	31596	3.194	11603
3	Jul 27 f	2188.0	<2.56	295.03	211.24	<0.32	128671	<0.32	1.590	4.848	14.900	5672.0	27504	2.828	6215.8
4	Jul 27 d	4460.1	4.281	343.64	210.52	<0.32	135607	0.389	3.099	10.753	27.723	7482.0	24565	4.642	7951.7
5	Aug 31 f	4026.5	4.959	280.14	219.93	<0.32	151470	0.342	3.117	9.687	27.314	7745.7	18329	4.164	7129.6
6	Aug 31 d	2385.5	3.032	290.64	190.17	<0.32	132193	<0.32	1.966	5.884	15.998	5639.0	32540	3.100	6773.7
6 dup	Aug 31 d dup	2361.8	2.661	292.99	191.23	<0.32	133122	<0.32	1.951	5.928	17.693	5667.4	32786	3.084	6879.0

Laboratory ID	Sample ID	Mn (mg/kg)	Mo (mg/kg)	Na (mg/kg)	Ni (mg/kg)	P (mg/kg)	Pb (mg/kg)	Rb (mg/kg)	S (mg/kg)	Si (mg/kg)	Sr (mg/kg)	Ti (mg/kg)	V (mg/kg)	Zn (mg/kg)
LOD (mg/L)**		0.029	0.001	0.007	0.007	0.029	0.001	0.122	0.011	0.045	0.001	0.004	0.038	0.048
LOD * average spl dilution factor (~mg/kg)		**9.28**	**0.32**	**2.24**	**2.24**	**9.28**	**0.32**	**39.04**	**3.52**	**14.40**	**0.32**	**1.28**	**12.16**	**15.36**
1	Jun 15 f	3438.4	0.938	11887	5.617	6440.2	23.823	<39.04	7992.2	2053.4	94.383	90.258	<12.16	70.506
2	Jun 15 d	4104.5	0.801	11738	3.913	8439.6	19.177	<39.04	7991.1	1647.7	105.32	69.283	<12.16	69.510
3	Jul 27 f	4302.9	0.535	5736.3	4.978	5885.2	24.671	<39.04	7462.2	1647.8	74.475	92.571	<12.16	65.484
4	Jul 27 d	3576.9	1.347	7015.8	10.779	5179.4	51.115	<39.04	7885.1	2544.1	79.780	172.77	16.140	99.242
5	Aug 31 f	3638.2	1.247	5216.3	9.381	4364.7	49.916	<39.04	8140.3	3010.6	80.993	169.41	15.304	100.66
6	Aug 31 d	3669.9	0.681	6840.2	7.616	4874.7	28.084	<39.04	7257.5	2220.2	75.455	104.54	<12.16	60.014
6 dup	Aug 31 d dup	3690.2	0.784	6894.3	5.724	4909.3	28.180	<39.04	7294.3	1983.2	75.924	100.73	<12.16	63.312

Table 4. Total Nitrogen of Composted Duckweed

Lab Sample Number	Sample ID	Total Nitrogen (% N)
1	Jun 15 f	1.823
1 dup	Jun 15 f dup	1.815
2	Jun 15 d	1.897
3	Jul 27 f	2.574
4	Jul 27 d	2.214
5	Aug 31 f	1.872
6	Aug 31 d	2.238

Discussion

Investigation of the samples revealed low levels of specimen variety and overall domination of *Wolffia spp*. The Shannon-Weiner index suggests that the diversity level of surface skimming in Loring Pond to be low, with the average index across the sample dates being H = 0.242. Our results can be compared to the 2018-2022 Wetland Hydrology Monitoring Report by the Minnesota DNR, where a variety of wetlands, including five shallow marshes, were monitored across the state. Taxonomic diversity was also calculated via Shannon-Weiner index, with calculated indices ranging from H = 2.7 – 3.6 (MN DNR Wetland Hydrology Monitoring Report 2023, p. 21). It is important to note that data collection is slightly different between our report and the DNR report, where diversity calculated by the DNR included organisms in and around the wetland and our diversity report is focused on the removal of duckweed during the surface skimming process.

Our observations of duckweed covered wetlands showing low levels of biodiversity are comparable to a study conducted in Scotland that found *Lemna minor* domination to decrease biodiversity. The woodland pond examined showed signs of impoverished macroinvertebrate biodiversity and species found were those who can thrive in O-depleted wetlands (Krivtsov et al., 2023). Another study conducted in Minneapolis found Cleveland Pond, a Lemna-infested urban pond, showed signs of alleviated eutrophic conditions and improved water quality following Lemna removal. Total Phosphorus concentration decreased slightly, and dissolved oxygen increased by 1019% from pre-removal period (Ronkainen, 2021). A study that focused on *Chara globularis,* a common, eutrophication-tolerant macroalga, found *L. minor* domination to inhibit growth of *C. globularis* and potentially lead to a gradual decline of its overall population (Van Onsem et al ., 2021).

Diversity can be seen to increase as the number of *Wolffia* observations decrease. Along with this pattern, total N, Cu, and Pb all increase inversely to number of *Wolffia* observations. This suggests that *Wolffia* inhibits biodiversity while also potentially absorbing these certain pollutants, where with less *Wolffia* available for uptake, levels of N, Cu, and Pb may increase. Utilization of composted duckweed seems viable with many heavy metals, including Be, V, Rb, and Cd falling below the detectible limit for the majority of the samples, if not in all. Pb was the only heavy metal to exceed limits for organic fertilizer of 27 mg/kg. This happened in 4 of the 7 results, with Pb reaching just over 51 mg/kg, though this amount never exceeding the limit allowed in soil (see table 5). The variability of the elemental analysis results show that further testing is needed to confirm the heavy metal content of composted duckweed from Loring Pond.

Some studies have found duckweed to be viable as fertilizer. One study that incorporated dry duckweed into agricultural fields found the application to be a sustainable source of N and P during their research (Kreider et al., 2019). Similarly, one research study found that duckweed mixed with fertilizer may be comparable to commercial fertilizer (F. Pulido, 2016). Duckweed amended treatments resulted in similar yield and biomass in sorghum and tomatoes compared to those grown with commercial fertilizer and were found to provide positive C and P soil residue (F. Pulido, 2016).

Table 5. Maximum concentrations (mg/kg) of heavy metals allowed in organic fertilizers, soil and plants in Brazil, the EU and the USA (de Souza et al., 2018).

		Cu	Zn	Ni	Cd	Cr	Pb
Organic fertilizer	Brazil[1]	no limit [5]	no limit	70	3	200	150
	EU[2]	33–60	109–347	4–44	0.2–0.7	5–56	5–18
	USA[3]	2–172	15–556	SL	0.1–0.8	1.1–55	1.1–27
Soil	Brazil [1]	49	46.5	21.5	<0.4	75	19.5
	EU[3]	50–140	150–300	20–75	1–3	100–150	50–300
	USA[3]	60–125	400–700	100	3–8	75–400	100–400
Plant	Brazil[1]	30	50	5	0.5	0.1	0.5
	EU[2]	no limit	no limit	no limit	0.2	no limit	0.3
	USA[4]	5–20	10–50	1–10	0.05–0.5	2	0.5–10

[1] MAPA (2006), COPAM (2011), Anvisa (1965, 1998); [2] European Commission (2006); [3] Alloway (1995); [4] Kabata-Pendias (2010); [5] No threshold established by the normative regulation.

In future studies, samples taken for biodiversity assessment should be preserved and surveyed more immediately to avoid potential deterioration of specimen. More samples should be taken more frequently, and more surveys should be completed to better assess the changes in diversity. Because chironomids were only noted in the August surveys, it could be that deterioration occurred in earlier samples. Although our results indicate low levels of diversity in surface skimming by-catch, numerous specimens were noted across all surveys, amounting to 15 genera total. Some larger specimens were removed during sampling, including some unidentified fish, crustaceans, and large leeches. In commercial, large-scale skimming of duckweed, our results suggest that removal would not be limited to duckweed and would include a variety of potential elements, including nutrients and other organisms.

Sources

Ekperusi, A. et al. Application of common duckweed (Lemna minor) in phytoremediation of chemicals in the environment: State and future perspective, Chemosphere, Vol. 223 (2019) https://www.sciencedirect.com/science/article/pii/S0045653519302413

State of Minnesota, Department of Natural Resources. Wetland Hydrology Monitoring: 2018-2022. https://files.dnr.state.mn.us/eco/wetlands/wetland-hydrology-monitoring-report-2022.pdf

Jewel, Md. Abu & Haque, Md & Khatun, Md & Rahman, & Jewel, Abu. A Comparative Study of Fish Assemblage and Diversity Indices in two Different Aquatic Habitats in Bangladesh: Lakhandaha Wetland and Atari River. Jordan Journal of Biological Sciences, Vol. 11, no. 4, pp 427–434 (2018).

Ronkainen, F. Investigation Effects of Lemna Removal on Oxygen and Phosphorus Recovery in Urban Ponds. MURAJ Vol. 4, Issue 5 (2021). https://pubs.lib.umn.edu/index.php/muraj/article/view/3665/2780

Krivtsov, V., Buckman, J., Birkinshaw, S. et al. Interactions of hydrology, geochemistry, and biodiversity in woodland ponds located in riverine floodplains: case study from Scotland. Environ Sci Pollut Res (2023). https://link.springer.com/article/10.1007/s11356-023-27890-6

Van Onsem, Stijn, and Ludwig Triest. "Trading Offspring for Survival: High Duckweed Cover Decreases Reproductive Potential and Stimulates Elongation in the Submerged Macrophyte Chara Globularis Thuillier." Hydrobiologia, vol. 848, no. 11, June 2021, pp. 2667–80. EBSCOhost, https://doi.org/10.1007/s10750-021-04577-y

Kreider, Andrew N. et al. “Duckweed as an Agricultural Amendment: Nitrogen Mineralization, Leaching, and Sorghum Uptake.” *Journal of environmental quality* vol. 48,2 (2019): 469-475. doi:10.2134/jeq2018.05.0207

Fernandez Pulido, Carlos Rolando. “DUCKWEED AS A SUSTAINABLE SOIL AMENDMENT TO SUPPORT CROP GROWTH, ENHANCE SOIL QUALITY, AND REDUCE AGRICULTURAL RUNOFF.” Department of Civil and Environmental Engineering. Pennsylvania State University. (2016). https://etda.libraries.psu.edu/files/final_submissions/12847

De Souza, Maria Eunice Paula. et al. “Rock Powder Can Improve Vermicompost Chemical Properties and Plant Nutrition: an On-farm Experiment.” *Communications in Soil Science and Plant Analysis*. 49: 1-12 (2018). 10.1080/00103624.2017.1418372.